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Potential susceptibility of Australian native plant species to branch dieback and bole canker diseases of *Phytophthora ramorum*

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Abstract

The invasive plant pathogen *Phytophthora ramorum* is the cause of considerable and widespread damage in nurseries, gardens and natural woodland ecosystems of the USA and Europe. In Australasia, Southern Africa and South America, it is considered to be a potentially significant plant pest of quarantine concern as it could cause biodiversity loss and severe economic losses to plant industries should it inadvertently be introduced. Branch dieback susceptibility was tested using a detached branch assay for 66 Australian native plant species sourced from established gardens and arboreta in California. Six of these species were

further tested for their susceptibility to bole cankers caused by *P. ramorum* using a sealed log assay. *Isopogon formosus* and *Eucalyptus denticulata* were identified as potentially highly susceptible Australian branch dieback hosts. Thirteen potentially tolerant Australian host species included *Banksia attenuata*, *B. marginata*, *Eucalyptus haemastoma*, *E. regnans*, *Pittosporum undulatum* and *Billardiera heterophylla*. *Eucalyptus regnans* was identified as a potentially highly susceptible bole canker host, while *E. diversicolor* and *E. viminalis* were considered potentially tolerant species to bole cankers caused by *P. ramorum*. *Phytophthora ramorum* was able to infect all 66 species, as confirmed by reisolation. These results extend the known potential host range for *P. ramorum*, confirm it as a possible threat to Australian plant industries and ecosystems and highlight additional associated hosts that are important in the global horticultural trade, native forests and plantation forestry.

Keywords: Invasive organism, Oomycete, Ramorum branch dieback and Sudden oak death.

Introduction

Phytophthora ramorum is an invasive plant pathogen causing considerable and widespread damage in nurseries, gardens and natural woodland ecosystems of the USA and Europe (Rizzo et al., 2005, Brasier & Webber, 2010). It is classified as a Category 1 plant pest risk to Australian plant biosecurity (i.e., a pest which if not eradicated would cause major damage to both natural ecosystems and plant industries/amenity flora) (Plant Health Australia, 2006) and is internationally recognised as a plant biosecurity threat. Australia, South Korea, Canada, the Czech Republic, Mexico, Taiwan and New Zealand have established quarantine policies and protocols against plant materials from areas known to have the disease (Kliejunas, 2010). Spread through the international nursery trade (Ivors et al., 2006, Brasier, 2008), *P. ramorum* has altered natural and forestry landscapes both in the south of the United

Kingdom (Brasier & Webber, 2010), western Scotland and Northern Ireland (Forestry Commission, 2011) and the Pacific coast of the USA (Oregon and California), where it is recognised as the causal agent of sudden oak death (Rizzo et al., 2005). Where it has been introduced into the ornamental plant trade, nursery and horticultural industries, the pathogen has caused considerable economic losses, resulting in the loss and destruction of many plant consignments and continued costs of surveillance and eradication (Dart & Chastagner, 2007). There have been two distinct introductions of *P. ramorum* into both Europe and North America, where it has continued to spread within the nursery industry on both continents (Ivors et al., 2006). Molecular evidence has demonstrated the transmission of the pathogen from nursery environments into natural ecosystems (Mascheretti et al., 2008, Goss et al., 2009).

Phytophthora ramorum causes a range of symptoms on more than 100 species of trees, shrubs and herbs (RAPRA, 2007, USDA-APHIS, 2010). Three distinct diseases are caused by *P. ramorum*: ramorum leaf blight, ramorum shoot dieback and sudden oak death (characterised by lethal bole cankers) (Hansen et al., 2005). The pathogen is spread primarily by aerial dissemination of sporangia and zoospores from foliar hosts which support high levels of sporulation, such as *Umbellularia californica* (California bay laurel) in Northern California and *Notholithocarpus densiflorus* (formerly *Lithocarpus densiflorus*; tanoak) in Northern California and Oregon (Goheen et al., 2002, Davidson et al., 2005) and *Rhododendron ponticum* and *Larix kaempferi* (Japanese larch) in the UK and Northern Ireland (Brasier et al., 2004, Brasier & Webber, 2010, Forestry Commission, 2011). Ramorum shoot dieback and sudden oak death cause severe and sometimes fatal infections of a number of hosts, particularly within the Ericaceae and Fagaceae(Davidson et al., 2003). This can greatly affect ecosystem structure and dynamics. Loss of keystone species such as *Quercus agrifolia* (coast live oak) and tanoak in Californian forests and *Fagus sylvatica*

(European beech) in Cornwall (Brasier et al., 2004) is postulated to have many detrimental effects on ecosystem health, including loss of habitat (Monahan & Koenig, 2006), modified fire risk (Metz et al., 2011), nutrient cycling disruption and changes in species distributions and dynamics (Cobb et al., 2010).

A number of native Australian plant species have been found to be either potential or naturally infected foliar or branch hosts of *P. ramorum*, when planted in the UK or California (RAPRA, 2007, Hüberli et al., 2008, Ireland et al., 2011, USDA-APHIS, 2010). *Correa* 'Sister *Dawn', Eucalyptus regnans, Isopogon cuneatus, I. formosus, Leptospermum scoparium, L. lanigerum* and *Melaleuca squamea*, have been identified as potentially highly susceptible Australian foliar host species (Ireland et al., 2011). Additionally, putative sporulating hosts have also been identified and include *Agonis flexuosa*, *Corymbia ficifolia*, *Eucalyptus haemastoma, E. delegatensis* and *E. viminalis* (Ireland et al., 2011). A number of other proven and associated host species of *P. ramorum* (see USDA-APHIS, 2010) have been introduced to Australia, often widely planted within home gardens (i.e. *Rhododendron* and *Camellia* spp.), as street trees (*Magnolia* spp.) or are planted in the Australian forestry industry (i.e. redwood, *Sequoia sempervirens*). Known branch dieback hosts native to Australia include *Acacia melanoxylon* and *Leptospermum scoparium* (Hüberli et al., 2008), while *Eucalyptus dalrympleana* has been identified as a potentially highly susceptible bole canker host (Moralejo et al., 2009). Given the wide and increasingly recorded host range of *P. ramorum*, consistent with the very large host ranges for generalist *Phytophthora* species (Hardham, 2005), it is expected that many Australian native plant species may be susceptible to the range of foliar, stem and bole canker diseases caused by this pathogen.

Results of branch wound inoculations of known hosts with *P. ramorum* have been shown to be generally congruent with field observations of ramorum shoot dieback symptoms (Dodd et al., 2005, Hansen et al., 2005). Likewise, log infection studies for testing susceptibility to

Phytophthora species are well-established and give a good reflection of potential aggressiveness under natural conditions when conducted on fresh, sealed logs (Brasier & Kirk, 2001, Hansen et al., 2005, Moralejo et al., 2009). Isolations of *P. ramorum* in Europe have also been made from several tree species in the field which were previously identified as potential hosts from artificial inoculations (Brasier et al., 2004), highlighting this methodology as a useful way of identifying potential hosts before establishment. In the study reported here, detached branch and log assays were used to assess the susceptibility of a range of commercially and ecologically important Australian native species to *P. ramorum*. The results of these tests are explored, related to concurrent work on foliar susceptibility, and quarantine and management recommendations for Australian and international plant biosecurity are discussed.

Materials and methods

Experimental design

Potential branch and bole canker susceptibility of detached branches and logs of native Australian plants were determined over the course of 18 experiments between April 2008 and September 2009 in Davis, California, USA (Table 1). Ten experiments were conducted during the summer (April – July) and five during the winter (November – January) to test branch dieback susceptibility. Bole canker susceptibility was tested over three experiments, all during the summer (August) of 2009.

Isolate and inoculum production

Isolate Pr-510 (Rizzo Lab collection) of the NA2 lineage (see Grünwald et al., 2009 for further detail regarding details and nomenclature of *P. ramorum* lineages), isolated from *Rhododendron* roots from a nursery in Sacramento County in 2006, was used in all

experiments (Table 2). It was shown to be highly pathogenic on the leaves of *U. californica* (California bay laurel) and *Rhododendron* cultivar 'Colonel Coen' and fast growing on both one-third-strength clarified V8 juice agar (1/3 V8; Campbell Soup Company; 66 ml of clarified V8 juice and 17 g of agar/L) and the *Phytophthora*-selective, pimaricin-ampicillin-rifampicin-pentachloronitrobenzene agar (PARP) (Jeffers & Martin, 1986) when compared with other isolates, including the commonly used isolate Pr-52 (Hüberli et al., 2008) (data not shown). The isolate was passaged at the start of each season through detached *Rhododendron* 'Colonel Cohen' leaves to maintain pathogenicity and maintained on PARP (Erwin & Ribeiro, 1996). This isolate was also used in a concurrent study of foliar susceptibility and sporulation potential (Ireland et al., 2011). Inoculum was cultured on 1/3 V8 agar at 20ºC in the dark and inoculum discs cut with a sterile cork borer from the margin of a 10 day old culture.

Six additional isolates of *P. ramorum* were used in the log experiments, one additional NA2, four NA1 and one EU1 lineage, all sourced from the University of California (UC) Davis, Rizzo Lab Collection (Table 2). Isolates were selected to represent a range of hosts and environments that *P. ramorum* has been isolated from in Northern California in order to potentially capture differences in pathogenicity related to the ecology and provenance of the isolate. All cultures were maintained as described previously, except that Pr-52 (CBS 110537; ATCC MYC-2436), Pr-155, Pr-461 and Pr-487 and *P. cinnamomi* isolate, P-541 (*Arbutus menzesii*, Santa Cruz, CA; Rizzo Lab Collection) were not passaged through *Rhododendron* leaves due to time restrictions. *Phytophthora cinnamomi*, a known root-rot and canker pathogen of oak trees and many Australian native plants (Hardham, 2005), was included in the study for comparative purposes to assess the aggressiveness of *P. ramorum* isolates.

Branch dieback experiments

Sixty-six Australian native plant species within 22 families and 40 genera were sourced from mature healthy plants in established gardens and arboreta in Northern California: San Francisco Strybing Arboretum, University of California (UC) Davis Arboretum, UC Berkeley Botanical Garden and UC Santa Cruz Arboretum. Species were selected from areas considered to have climates suitable for *P. ramorum* survival in Australia. This was based on observations of suitable climate for the pathogen in the USA and Europe and a preliminary CLIMEX (Sutherst & Maywald, 1985) model developed by E.A. Pinkard and I.W. Smith (CSIRO Hobart and University of Melbourne, *personal communication*) based on the parameters published by Venette and Cohen (2006), as well as for the plant species ecological and economic importance to Australian plant industries. Experimental replication was limited by the number of individual plants kept in the botanical collections. Individuals of a species were duplicated where possible from different locations or accessions, to give a total of 128 individual Australian plants tested. Four to twenty-four hosts were tested in any one experiment based on collection from common locations and ease of management (Table 1). Four branches of each individual plant were inoculated in the summer studies and ten branches in the winter studies. The known susceptible host *R.* 'Colonel Coen' (kept in controlled environment facilities and greenhouses at UC Davis) was used as a positive control species in all experiments to confirm the pathogenicity of *P. ramorum*. Likewise, *U. californica* (sourced from a private garden in Davis, California), was included in one experiment (B-15; Table 1) as an additional positive control species.

Branches were collected two days before they were inoculated and the cut bases kept in deionised water. Before inoculation, branches of approximately 2 to 10 mm in diameter (depending on the species, e.g. smaller diameter branches were used for *Hardenbergia violaceae*, which has narrow twining vines, and *Leptospermum* species which have small

176 branches) were stripped of excess leaves, trimmed to 20 to 30 cm in height and kept in glass
177 flasks of sterile deionised water sealed with Parafilm (Pechiney Plastic Packaging) to reduce
178 evaporation for the duration of the experiment. Young branches with green bark were tested
179 for *Eucalyptus leucoxylon*, *E. sideroxylon* and *Pittosporum undulatum* during winter
180 experiments.
181 Plants from the UC Santa Cruz Arboretum were visually inspected and treated with
182 insecticide before shipping to UC Davis, in accordance with California's Light Brown Apple
183 Moth (*Epiphyas postvittana*) quarantine regulations at the time. Insecticide treatments were
184 made up in water with either DiPel (*Bacillus thuringiensis*; Abbot Laboratories) at 1.6 to 3.9
185 ml liter^{-1} of water and Vegol (canola oil; Lilly Miller Brands) at 3.9 to 19.5 ml liter^{-1} or
186 Sunspray Oil (Paraffinic Oil; Sun Refining & Marketing Co.) at 6.5 ml liter^{-1} for the summer
187 inoculations, and with Conserve SC (Spinosad; Dow Agrosciences LLC) at 1.7 ml liter^{-1} and
188 Bonide All Seasons Spray Oil (Petroleum Oil) at 10 ml liter^{-1} for the winter inoculations.
189 These species were rinsed well with deionised water upon arrival in Davis to remove the
190 insecticides. A preliminary test (data not shown) showed that inseticide application did not
191 significantly influence host susceptibility to *P. ramorum* for *Agonis flexuosa*, *Corymbia*
192 *ficifolia*, *Eucalyptus sideroxylon*, *E. viminalis* and *R.* 'Colonel Coen'. It was therefore
193 assumed that insecticide application did not have a significant effect on plant material of all
194 species collected and treated from this site.
195 Susceptibility to branch dieback was tested using a detached branch assay adapted from a
196 method devised by Hüberli et al. (2008). Using a sterile 32 gauge hypodermic needle a
197 wound was created through the bark, approximately 10 cm from the acropetal end of the
198 branch. An inoculum disc, 3 mm in diameter, was placed mycelium surface down on the
199 wound and the inoculation point carefully wrapped with a layer of Parafilm. One branch from
200 each individual plant in each experiment was inoculated with a sterile 1/3 V8 disc as a

negative control. Flasks with branches were placed randomly into large plastic boxes with transparent lids and sprayed down with deionised water to prevent desiccation and maintain humidity. These plastic boxes were then transferred to a controlled environment facility (PGR15, 2002; Conviron Controlled Environment Ltd) with cyclic regimes of 20 to 25°C and 16 h photoperiod during summer and 15 to 20°C and 12 h photoperiod during winter. Chambers were checked regularly throughout the experiment and the sides of the boxes were sprayed when necessary with deionised water to ensure high humidity.

Ten days after inoculation, the outer bark surrounding the inoculation site was carefully scraped off with a scalpel and the entire lesion (if present) exposed. Lesion length and presence or absence of branch girdling were recorded. Two to four pieces (4 to 20 mm^2) of branch tissue from the margins of the lesions or site of inoculation were plated onto PARP to confirm infection by *P. ramorum*. The reisolation data were used to assign proportions of branch infection (presence or absence of infection of the branch) and infection potential (number of plated tissue pieces with *P. ramorum* isolation/total number of plated tissue pieces) measures to each species.

A species reaction to infection was assigned to different categories of susceptibility according to the extension of necrosis, modified from classes defined by Kaminski and Wagner (2008). Species were considered to be: (i) tolerant to an isolate/species if the mean lesion size did not exceed the inoculation point (approximately 2 mm diameter); (ii) of low susceptibility when lesion size was greater than 2 mm but less than 15 mm; (iii) moderately susceptible between 15 and 30 mm; and (iv) highly susceptible above 30 mm lesion length.

Bole canker experiments

Six Australian native tree species, *Acacia dealbata, Eucalyptus denticulata, E. diversicolor, E. globulus, E. regnans* and *E. viminalis*, were tested for their susceptibility to bole canker

disease caused by *P. ramorum*. The species were selected based upon availability in the field and were collected over three experiments in order to be logistically manageable. The known susceptible host *N. densiflorus* was used as a positive control to confirm pathogenicity of *P. ramorum*. All species of *Eucalyptus* logs were sourced from mature healthy plants from the UC Santa Cruz Arboretum. *Acacia dealbata* and additional *E. globulus* material were sourced from Mills Creek Open Space Preserve, while *N. densiflorus* logs were sourced from Los Trancos and Monte Bello Open Space Preserves in the Midpeninsula Regional Open Space District of the San Francisco bay area. To allow for comparability between species in different experiments, *E. viminalis* was included in both experiments conducted on logs from the UC Santa Cruz arboretum and *E. globulus* was collected from both sites. All inoculations were conducted within a 7-day period to reduce variability between the three experiments. Three to nine individual trees of each species were tested, with one to three replicate logs per tree. Logs of each individual, 1 to 1.2 m long by 8 to 20 cm diameter, were collected in the morning to early afternoon one to two days prior to inoculation. The bottom and top ends of the logs were marked and immediately sealed with a water-based wax emulsion sealant (Waxlor, Willamette Valley Co.) to retard drying.

At the beginning of each experiment bark thickness per log and percent bark moisture content per tree were recorded. Bark moisture content was calculated by comparing the wet and dry weight of five 10 mm diameter plugs of bark from an additional sealed log section. Plugs were removed using a cork borer, immediately weighed, dried at 60°C for 48 h in a drying oven and reweighed.

Log susceptibility studies followed the methods of Brasier and Kirk (2001), with minor modifications. A 6 mm diameter hole was punched through the bark to the wood surface using a sterile cork borer and the bark plug removed. A 6 mm diameter plug from the margin of an actively growing colony of *P. ramorum, P. cinnamomi,* or a plug of 1/3 V8 agar

(negative control) was then inserted with aerial mycelium face down and the bark plug replaced. One log of each species was inoculated directly onto the surface of the bark by gently scraping an area of approximately 6 mm diameter to remove dirt and by placing the inoculum plug mycelium side down to assess whether infection could occur directly through the bark without wounding. Moist cotton wool was placed over the inoculation site and covered with a piece of aluminium foil secured by adhesive PVC tape. Nine inoculation points were arranged on the log along three transverse lanes separated by approximately 25 cm along the length of the log and each inoculation point on the lane by approximately 10 cm (three points at each lane). Each log was inoculated one to two days after collection from the field with seven different *P. ramorum* isolates (Table 2), one *P. cinnamomi* isolate as a positive control and one plug of 1/3 V8 agar to act as a negative control. Inoculated logs were sprayed down with deionised water, placed in clear plastic bags to keep moist and placed randomly into large temperature controlled chambers (PGR15, 2002; Conviron Controlled Environment Ltd, Canada) set to a continuous 20°C with a 16 hour photoperiod for 36 to 40 days.

Logs were destructively sampled by removing the outer bark surrounding each inoculation point with a drawknife. Any lesion or stained necrotic area was then quickly outlined with a marker pen and traced onto clear plastic sheets. The resulting images were scanned, lesion areas calculated using the image analysis software ASSESS v1.01 (APS Press) and lesion length and width measurements were made from photocopies of the traced lesions. Two to eight pieces (6 to 20 mm^2) of woody tissue from lesion margins were plated onto PARP to confirm infection by *P. ramorum* and *P. cinnamomi*.

A species reaction to infection by *P. ramorum* and *P. cinnamomi* was assigned to different categories of susceptibility according to the size of the lesion area, as defined by Moralejo et al. (2009). Species were considered to be: (i) tolerant to an isolate/species if the mean lesion

area did not differ significantly from those in the negative controls; (ii) of low susceptibility when mean lesion area was significantly larger than those in the negative controls, but below 10 cm^2; (iii) moderately susceptible between 10 and 20 cm^2; and (iv) highly susceptible above 20 cm^2.

Statistical analysis

Statistical analysis was performed with SAS software (version 9.1; SAS Publishing). Incidence of disease and branch or log infection were analysed using a binomial generalised linear model with a logit link. Lesion lengths, width and area were analysed using a log + 0.01 transformation and a general linear model. Predictions of the means generated by the models are presented in the branch results for all parameters except branch girdling (Table 3), while results from the bole canker (log) experiments are presented as the raw means ± the standard errors with the results of the statistical models due to the small experimental size (Fig. 2). Paired t-tests were conducted using JMP software (version 8.0, SAS Publishing) to test significance of branch age using the t-test for unbalanced variances for individual plants of *P. undulatum* and balanced variances for individual plants of *E. leucoxylon* and *E. sideroxylon*. Paired t-tests and the Students t-test were used to compare means between lesions formed on logs of *E. globulus* collected from different sites.

Results

Branch susceptibility

Isopogon formosus and *Eucalyptus denticulata* were identified as potentially highly susceptible Australian branch dieback hosts, with mean lesion lengths of 51.8 and 42.7 mm, respectively (Table 3). The positive control species were also highly susceptible, with the greatest mean lesion length of 91.8 mm in *R.* 'Colonel Coen'. Five potentially moderately

susceptible branch dieback hosts were *Hardenbergia violaceae*, *Eucalyptus cneorifolia*, *Nothofagus cunninghamii*, *E. viminalis* and *E. sideroxylon*, with mean lesion lengths ranging from 15.6 to 19.4 mm (Table 3). Thirteen potentially tolerant Australian host species were identified (Table 3) and included *Banksia attenuata*, *B. marginata*, *Eucalyptus haemastoma*, *E. regnans*, *Pittosporum undulatum* and *Billardiera heterophylla* (formerly *Sollya heterophylla*). The majority of species were of low susceptibility (46/66), including all of the conifers tested (Table 3).

Lesion lengths of noninoculated branches rarely exceeded the inoculation point (approximately 2 mm) and were smaller ($P < 0.0001$) than the lesions of inoculated branches. Ranges of lesion lengths varied widely, particularly for those species in the moderate to high susceptibility categories. More than half of the Australian species tested (43/66), and all of the moderate and highly susceptible species, had some proportion of girdling among the branches inoculated (Table 3). Inoculated *R.* 'Colonel Coen' and *U. californica* branches were all found to be infected and produced consistently large lesions across all experiments, confirming the virulence of the isolate (Table 3).

All species in the branch susceptibility study became infected with *P. ramorum*. Analyses of branch infection and infection potential were conducted on inoculated material only, as *P. ramorum* was never isolated from any of the control branches. Twenty-eight Australian species and both of the positive control species, in which all branches were infected when inoculated with *P. ramorum* (Table 3), were excluded from further analyses of branch infection. Likewise, ten Australian species and the positive controls *R.* 'Colonel Cohen' and *U. californica*, in which all plated branch pieces were infected with *P. ramorum* (Table 3), were excluded from further analyses of infection potential. Most species (61/66) had more than 70% of their branches infected, with infection potential recoveries above 60%. *Callitris*

rhomboidea, *Lomatia myricoides*, *P. undulatum*, *B. heterophylla* and *Tasmannia lanceolata* had less than 65% of inoculated branches infected.

Lesions formed on young green branches of *E. leucoxylon* ($P = 0.04$) and *E. sideroxylon* ($P < 0.05$) were larger than those formed on mature (more woody) branches (Fig. 1a). Lesions on younger branches of *P. undulatum* did not differ from those of mature branches (Fig. 1a). The proportion of branches infected did not differ between branches of different ages for *E. leucoxylon* and *E. sideroxylon* (Fig. 1b). No infection was recorded on young branches of *P. undulatum*, while 80 % of mature branches were infected ($P = 0.0002$) (Fig. 1b).

Season was not found to significantly affect branch infection, infection potential or length of lesions.

Bole canker susceptibility

Eucalyptus regnans was ranked as a potentially highly susceptible Australian species to bole canker development when inoculated with *P. ramorum*, with an overall mean lesion area of 55.4 cm^2 (Table 4), and means ranging from 32.8 to 77.1 cm^2 among the isolates tested ($P < 0.05$) (Fig. 2). *Eucalyptus denticulata* was identified as a potentially low susceptibility bole canker host overall with a mean lesion area of 9.6 cm^2 (Table 4), and mean lesion areas ranging from 5.2 to 14.8.cm^2 among the *P. ramorum* isolates tested (Fig. 2). Lesions caused by different *P. ramorum* isolates on *E. denticulata* fell into two groups of low and moderate susceptibility ($P < 0.05$). Those isolates which formed larger lesions on *E. denticulata*, Pr-487, Pr-500, Pr-510 and Pr-514, were not different to lesions areas associated with inoculations of the *P. cinnamomi* isolate included in the study (Fig. 2). *Acacia dealbata* (overall mean 5.5 cm^2, 4.9 to 6.1 cm^2 among isolates) and *E. globulus* (overall mean 6.8 cm^2, 3.8 to 8.8 cm^2 among isolates) were identified as being of potentially low susceptibility (Table 4; Fig. 2). *Eucalyptus diversicolor* and *E. viminalis* were identified as potentially

tolerant species, as the lesions which developed when inoculated with *P. ramorum* were not significantly different to the negative control inoculation (Fig. 2). *Notholithocarpus densiflorus* was ranked as highly susceptible, with an overall mean lesion area of 59 cm^2 and mean lesions areas ranging from 25.95 to 84.9 cm^2, confirming the virulence of the isolates (Table 4; Fig. 2).

With the exception of *E. diversicolor*, lesion areas which developed following inoculation with the positive control *P. cinnamomi* isolate were consistently larger ($P < 0.0001$) than those that developed following the negative control inoculations, confirming its virulence (Fig. 2). Lesion areas of these *P. cinnamomi* inoculations were approximately 2 to 5 times larger ($P < 0.0001$) than any lesions which developed following inoculation by any of the seven *P. ramorum* isolates for *E. globulus*, *E. regnans* and *E. viminalis* and *N. densiflorus*. Lesion area did not differ significantly between *P. ramorum* isolates and the *P. cinnamomi* inoculations for *A. dealbata* and *E. denticulata* (Fig. 2).

Mean lesion lengths and widths after inoculation with *P. ramorum* isolates followed similar trends to that of lesion area for all species (Table 4). Among the Australian trees, the greatest mean lesion length (15.8 cm) and width (4.6 cm) occurred in *E. regnans* (Table 4).

All species were able to be infected with *P. ramorum* (Table 4). Infection potential (IP) and log infection (LI) were consistently the lowest for the potentially tolerant species *E. diversicolor*, with 20 to 60 % of logs infected and infection potential less than 36 % for all isolates tested (Table 4). *Phytophthora ramorum* was more readily reisolated (infection potential, IP) and more readily infected (log infection, LI) from all of the other Australian species. Between 40 to 100 % of logs were infected and pathogen recovery between 26 to 80 % for *A. dealbata*, *E. denticulata*, *E. globulus*, *E. regnans* and *E. viminalis* (Table 4). *Notholithocarpus densiflorus* on the other hand had consistently high levels of infection potential (71 to 100%) and log infection (>85%) (Table 4).

There were no significant differences for all of the parameters between sites for *E. globulus* and isolates and isolates*species interactions for all of the species tested (data not shown). No correlations between bark thickness or bark moisture content and lesion area, length, width or infection potential were found. The thinnest bark was in *A. dealbata* (1.6 to 3.5 mm), followed by *N. densiflorus* (3.1 to 14.5 mm), *E. regnans* (3.6 to 4.6 mm), *E. globulus* (3.8 to 9.3 mm), *E. diversicolor* (4 to 11 mm), *E. viminalis* (4.9 to 12.0 mm) and *E. denticulata* (5.3 to 11.3 mm). The positive control species *N. densiflorus* had the greatest bark moisture content (7 to 28.7 %), followed by *E. globulus* (10.3 to 26.1 %), *E. viminalis* (12.8 to 23.8 %), *E. denticulata* (16.3 to 18 %), *E. diversicolor* (14.6 to 17.2 %), *E. regnans* (9.1 to 11.6 %) and *A. dealbata* (4.6 to 11.3 %).

All species were capable of being infected by both *P. ramorum* and *P. cinnamomi* during the nonwounded log inoculations, although much smaller lesions were produced on average (data not shown). Lesions produced on each log of each species by the isolates of *P. ramorum* were smallest for *E. viminalis* (0.54 ± 0.22 cm^2), followed by *Acacia dealbata* (0.55 ± 0.06 cm^2), *E. globulus* (0.63 ± 0.16 cm^2), *E. denticulata* (1.63 ± 0.43 cm^2), *N. densiflorus* (9.38 ± 3.82 cm^2), *E. diversicolor* (11.24 ± 2.97 cm^2) and *E. regnans* (34.51 ± 4.81 cm^2). The lesions produced on *E. diversicolor* by *P. ramorum* isolates when nonwounded may not be associated with *P. ramorum* infection given that only one of the seven isolates (Pr-155) was reisolated from the log at a 25% recovery rate (IP). Every isolate of *P. ramorum* was reisolated from *E. regnans* and *A. dealbata*, two out of seven from *E. viminalis* and five out of seven for *N. densiflorus*. Independent isolate data were not recovered for *E. denticulata* and *E. globulus*.

Discussion

Our study shows that branch dieback and bole canker lesion development caused by infection by *P. ramorum* may occur on Australian plant species. Potentially highly susceptible ramorum shoot dieback hosts were identified as *I. formosus* and *E. denticulata*, while *E. cneorifolia*, *E. sideroxylon*, *E. viminalis*, *H. violaceae* and *N. cunninghamii* were identified as being of potentially moderate susceptibility. *Eucalyptus regnans* was the only Australian species tested in this study which was identified as a potentially highly susceptible bole canker host, with lesions similar to those produced on the highly susceptible tanoak.

As with foliar studies of these same species (Ireland et al., 2011), in the current study the majority of species were of low susceptibility and potentially tolerant species were identified. *Isopogon formosus* has been identified as both a highly susceptible foliar (Ireland et al., 2011) and branch dieback host. On the other hand, *E. regnans*, while identified as highly susceptible in foliar and log experiments with *P. ramorum*, was not highly susceptible to branch dieback infections. This demonstrates the range of susceptibility of hosts to the different diseases caused by *P. ramorum* in the field. Some species such as tanoak are known to support all three types of diseases, while California bay laurel is almost exclusively a foliar host and coast live oak almost exclusively a bole canker host (Davidson et al., 2003).

Our studies confirm the susceptibility of *Acacia melanoxylon* and *Leptospermum scoparium* to ramorum branch dieback (Hüberli et al., 2008). Lesions recorded by Hüberli et al. (2008) were larger for both of these species and *Eucalyptus globulus*. While a greater success of pathogen reisolation was recorded in our studies for *L. scoparium* and *E. globulus* , our reisolation of *P. ramorum* from symptomatic tissue of *A. melanoxylon* was less successful. Girdling, previously not observed by Hüberli et al. (2008), was observed for branches of *A. melanoxylon* and *L. scoparium* in our studies. Girdling is considered to be epidemiologically

important as this symptom can lead to entire branch tip mortality as vascular tissues are completely occluded and no water or nutrient flow can occur.

While susceptibility classes were predicted based on the lesion length response of a species following inoculation, as has been employed by other authors (Kaminski & Wagner, 2008), presence of girdling was also found to be indicative of potential susceptibility classes. All of those species classified as moderate to highly susceptible branch dieback hosts in our studies had high levels of branch girdling. Levels of girdling increased from the moderately (> 30%) to the highly (> 55%) susceptible Australian species, with the positive control species recording the highest levels of girdling (> 94%). Only four of the low susceptibility hosts had girdling of more than 39% of branches (*E. pauciflora*, *A. flexuosa*, *B. rubiodies* and *Hakea rostrata*), while the majority only had girdling levels between 3 to 27% (25/46). Approximately half of the species classified as tolerant had no girdling and the majority of those which did had low levels (4 to 11%). However, the tolerant species *E. regnans* had a 40% presence of girdling of inoculated branches and 100% reisolation of *P. ramorum*. Given the epidemiological importance of girdling we recommend that species with greater than 50% of presence of branch girdling be elevated to high susceptibility classes, while those with 30% to 50% levels of branch girdling be elevated to moderate susceptibility classes. Under this classification system *E. pauciflora*, *A. flexuosa*, *B. rubiodies*, *H. rostrata* and *E. regnans* were considered to be moderately susceptible ramorum branch dieback hosts.

The present study revealed that the living inner bark (phloem) of *E. regnans* is highly susceptible to *P. ramorum*. These lesion sizes were comparable to those recorded for the highly susceptible *N. densiflorus* both in our study and in a separate study by Hansen et al. (2005), and comparable to mean lesion sizes reported by Brasier et al. (2006) on the highly susceptible *Fagus sylvatica* . Lesion widths were also comparable between *N. densiflorus* and *E. regnans* in our studies. Epidemiologically, wider lesions suggest a higher risk for branch

girdling and subsequent tree mortality to occur (Moralejo et al., 2009). Because lesion sizes on *E. regnans* were comparable to those of *N. densiflorus* and *F. sylvatica*, two of the most susceptible natural hosts of *P. ramorum*, it is thought that lethal bole cankers may develop on *E. regnans* if trunks were to become infected. Should *P. ramorum* establish in Australia such infection may occur via putative sporulating hosts such as *E. viminalis* and *N. cunninghamii* (Ireland et al., 2011), which co-occur with *E. regnans* in natural forests of south-eastern Victoria (Boland et al., 2006) and are native to areas which have been identified as having climatic conditions conducive to *P. ramorum* growth and survival (Ireland et al., 2011). Only one other Australian species, *E. dalrympleana*, has been confirmed as a potential bole canker host for *P. ramorum* (Moralejo et al., 2009). Mean lesion area and range of lesion areas recorded by Moralejo *et al*. (2009) for *E. dalrympleana* are comparable to lesion sizes we found for *E. regnans* in our studies. All of the other *Eucalyptus* species that we tested, including the global plantation species *E. globulus* , were predicted to be of low susceptibility or potentially tolerant to bole canker infections of *P. ramorum*. It is important to note that differences in *E. globulus* susceptibility, particularly in regards to infection potential, have been found between branch and foliar susceptibility studies on detached material (Hüberli et al., 2008, Ireland et al., 2011). Larger than expected lesions following inoculation with *P. cinnamomi* on *E. regnans* were also recorded in our study. This confirms known susceptibility of this species to *P. cinnamomi* in Australia when grown offsite (Harris et al., 1983) and adds to the confidence of the method to provide accurate susceptibility data. Variation in susceptibility of provenances of *E. regnans* to *P. cinnamomi* has been observed in Victoria (Harris et al., 1983), with higher rates and severity of disease occurring when planted outside of their natural range on potentially less suppressive soils and in conducive climates (Weste & Marks, 1987).

Green, juvenile branches of *Eucalyptus leucoxylon* and *E. sideroxylon* were also shown to be considerably more susceptible to *P. ramorum* infection in our study. On the other hand, lesion size did not differ between branches of different ages for *P. undulatum* and no infection was recorded on younger branches, indicating a potential hypersensitive response and potential tolerance or resistance to *P. ramorum* when host tissue is younger. This indicates that the phenological condition of the host at the time of transmission of the pathogen may affect its overall susceptibility, and that this is likely to be variable amongst different species (Dodd et al., 2008). Therefore, while caution should be taken when extrapolating to a whole species susceptibility, the potential for putative tolerant or low susceptibility hosts to be infected under different site and climatic conditions should not be underestimated.

Susceptibility studies, particularly those conducted on detached plant material are naturally fraught with difficulties, especially when it comes to interpretation of results. Log inoculation methods are well established and are considered to provide a realistic estimate of potential susceptibility to *P. ramorum* (Hansen et al., 2005). However, the method is cumbersome and subject to seasonal variability, with greater susceptibility recorded during the summer in previous studies (Brasier & Kirk, 2001, Hansen et al., 2005, Moralejo et al., 2009). Despite our small data set, obtained over the summer, the lesion sizes and the lack of variability amongst isolates were similar to the results of previous log inoculation experiments with *P. ramorum* (Hansen et al., 2005, Moralejo et al., 2009) and other *Phytophthora* species (Brasier & Kirk, 2001)..

In natural situations zoospores or sporangia (not mycelia) are believed to be responsible for infections and must gain entrance through the outer bark (Moralejo et al., 2009). While successful infections were observed using non-wounded mycelial inoculations of logs of the same log species tested here, our results should be interpreted carefully until they are

confirmed by whole plant inoculation with zoospores, which is considered to provide the best prediction of natural susceptibility to the range of diseases caused by *P. ramorum* (Hansen et al., 2005). Nevertheless, our results indicate that those species that did not develop extensive necrosis are unlikely to be hosts, given the invasive nature of the inoculation method and the ability of the host to prevent its spread under these conditions.

No interactions between disease severity or infection potential and isolates were found to occur among the isolates used in our study, although some species*isolate interactions were observed. Studies by Brasier (2003) have indicated that isolates of the A1 mating type (EU1) are likely to be more aggressive in log inoculations than those of the A2 mating type (NA1) and Hüberli et al. (2011) found isolates originating from Santa Cruz county in California were more pathogenic on coast live oak seedlings than those which originated in two other counties tested. On the other hand, Moralejo et al. (2009) found no differences in aggressiveness among the isolates they tested in a similar study, including between different mating types, and no significant differences in aggressiveness amongst isolates and mating types have been found in foliar studies (Denman et al., 2005, De Dobbelaere et al., 2010). Similar to our studies, Kaminski and Wagner (2008) and Denman et al. (2006) have found preliminary evidence of host species*isolate interactions for particular host species, indicating significant differences among isolates may occur only on an individual host species basis. Unique differences in growth (Grünwald et al., 2009) and sporulation potential (McDonald & Grünwald, 2007) have been observed among different genotypes of *P. ramorum*. Therefore, further work into their comparative aggressiveness, utilising more isolates under common conditions and with a range of hosts, will be crucial when developing adequate quarantine regulations.

Multiple parameters of disease must be considered when attempting to determine the overall susceptibility of a given species to *P. ramorum*. We believe infection potential to be more

appropriate for assessing potential susceptibility of logs in particular, as the parameter allows one to potentially assess how readily *P. ramorum* can be reisolated from infected material and in doing so may indicate hosts with more hostile tissues to *P. ramorum* when not readily reisolated. To support this, we consistently found that species we believed to be of lower susceptibility to have lower levels of infection potential, while overall log infection did not vary as greatly. Likewise, Moralejo et al. (2009) noticed that colonies that formed on selective agar from tissue derived from small lesion areas were inhibited, while those from larger lesions were usually more diffuse and grew faster. This was also observed for less susceptible species in foliar susceptibility studies of Australian species (Ireland et al., 2011). This study has clearly shown that Australian plant species from a range of families and genera are potentially susceptible to ramorum branch dieback and sudden oak death diseases caused by *P. ramorum*. Transmission of *P. ramorum* from identified foliar sporulating hosts (Ireland et al., 2011) onto these potential branch and bole canker hosts could, in a disease conducive environment, result in altered ecosystem structure and dynamics, biodiversity loss and serious economic losses in global forest and horticulture industries which utilise susceptible Australian species.

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663 **Table 1.** Details of experiments used to test the susceptibility of Australian
664 native plant species to branch dieback and bole canker diseases caused by
665 *Phytophthora ramorum*

Experiment	Year	Month [a]	Collection site [b]	Season [c]	No. of species [d]
Branch dieback susceptibility					(66)
B-01	2008	April	UCD	Summer	8
B-02	2008	May	SFBG	Summer	7
B-03	2008	May	SFBG	Summer	6
B-04	2008	May	SFBG	Summer	11
B-05	2008	June	UCD	Summer	6
B-06	2008	June	UCB	Summer	13
B-07	2008	June	UCSC	Summer	15
B-08	2008	June	UCSC	Summer	14
B-09	2008	July	UCSC	Summer	12
B-10	2008	Nov	UCD	Winter	14
B-11	2008	Nov	SFBG	Winter	24
B-12	2008	Dec	UCB	Winter	12
B-13	2009	Jan	UCSC	Winter	17
B-14	2009	Jan	UCSC	Winter	23
B-15	2009	May	UCSC	Summer	4
Bole canker susceptibility					(6)
L-01	2009	Aug	UCSC	Summer	4
L-02	2009	Aug	USCS	Summer	2
L-03	2009	Aug	MPROSD	Summer	2

[a] Month experiment was started.

[b] MPROSD = Midpeninsula Regional Open Space District; SFBG = San Francisco Botanical Garden & Strybing Arboretum; UCD = University of California (UC) Berkeley Gardens; UCD = UC Davis Arboretum; UCSC = UC Santa Cruz Arboretum.

[c] April to August = Summer, November to February = Winter.

[d] Total number of host species in brackets for all experiments for branch or log susceptibility. Species were replicated over seasons and some with multiple individual plants tested per species. Positive control species *Rhododendron* 'Colonel Coen' was included in all branch susceptibility experiments and *Umbellularia californica* was included in experiment B-15. *Notholithocarpus densiflorus* was included as a positive control species for log susceptibility experiments in L-03.

677 **Table 2.** *Phytophthora ramorum* isolates used in log inoculation tests.

Isolate [a]	Lineage [b]	Mating type	Host	Location
Pr-52	NA1	A2	*Rhododendron* sp.	Nursery, Santa Cruz, CA
Pr-155	NA1	A2	*Notholithocarpus densiflorus*	Woodland, Santa Clara, CA
Pr-461	NA1	A2	*Quercus chrysolepis*	Woodland, Humboldt, CA
Pr-487	NA1	A2	*Umbellularia californica*	Woodland, Sonoma, CA
Pr-500	NA2	A2	*Rhododendron* sp. - shoots	Nursery, Sacramento, CA
Pr-510	NA2	A2	*Rhododendron* sp. - roots	Nursery, Sacramento, CA
Pr-514	EU1	A1	*Rhododendron* sp. - bait leaf	Stream, Humboldt, CA

678 [a] All isolates sourced from the University of California, Davis, Rizzo Laboratory Collection.

679 [b] NA1 = North American genotype 1; NA2 = North American genotype 2; EU1 = European

680 genotype 1. See Grünwald et al. (2009).

681 **Table 3.** Potential susceptibility of detached branches of Australian plant species inoculated
682 with *Phytophthora ramorum*, presented in descending order of greatest mean lesion length,
683 with levels of branch infection and infection potential as measures of reisolation.

Susceptibility group and species [a]	Plants [b]	Lesion length (mm) [c]		Branch Infection [d]	Infection Potential [e]
Positive control species					
High Susceptibility (> 30 mm)					
Rhododendron 'Colonel Coen'	(all)	91.8	(16.6 - 508.5) #	all	all^
Umbellularia californica	1 (1)	44.2	(21.5 - 91) #	all	all
Australian species					
High Susceptibility (> 30 mm)					
Isopogon formosus	3 (2)	51.8	(8.9 - 301.1) #	all	0.93
Eucalyptus denticulata	1 (4)	42.7	(6.3 - 290.4) #	1.00	0.73
Moderate Susceptibility (15 - 30 mm)					
Hardenbergia violaceae	3 (4)	19.4	(3.1 - 121.6) #	0.90	0.85
Eucalyptus cneorifolia	1 (1)	18.9	(3 - 119.8) #	0.90	0.90
Nothofagus cunninghamii	1 (2)	17.6	(2.8 - 111.7) #	all	0.96
Eucalyptus viminalis	2 (4)	16.4	(2.6 - 102.9) #	all	0.96
Eucalyptus sideroxylon	2 (4)	15.6	(2.5 - 96.9) #	all	0.97
Low Susceptibility (2 - 15 mm)					
Acacia dealbata	1 (1)	14.9	(2.1 - 103.4) #	all	0.90
Eucalyptus diversicolor	1 (3)	13.9	(2 - 94.8) #	all	0.95
Brachychiton populneus	3 (2)	13.2	(2.4 - 71.1) #	all	all
Eucalyptus pauciflora	3 (2)	12.7	(2.3 - 69.2) #	all	0.99
Acacia melanoxylon	1 (1)	11.1	(1.6 - 77.1) #	0.76	0.60

Eucalyptus laeliae	1 (2)	10.1	(1.6 - 63.7) #	0.92	0.89
Phyllocladus aspleniifolius	1 (2)	10	(1.6 - 61.3) #	all	0.81
Nothofagus moorei	2 (2)	9.6	(1.6 - 57.9) #	0.98	0.97
Eucalyptus leucoxylon	4 (2)	9.1	(1.5 - 56.2) #	0.95	0.93
Corymbia ficifolia	2 (5)	8.5	(1.4 - 50.5) #	0.96	0.85
Agonis flexuosa	4 (6)	8.4	(1.4 - 50.6) #	0.83	0.76
Hedycarya angustifolia	1 (2)	8.3	(1.3 - 51.2) #	0.83	0.69
Eucryphia lucida	3 (2)	7.4	(1.2 - 44.6)	0.89	0.81
Eucalyptus camaldulensis	2 (2)	6.8	(1 - 44.9) #	0.89	0.78
Dodonea viscosa	2 (3)	6.5	(1.2 - 35.7) #	all	0.98
Eucalyptus saligna	1 (2)	6.3	(0.9 - 44)	all	0.88
Polyscias sambucifolia	2 (1)	6.2	(0.8 - 50.2) #	all	all
Atherosperma moschatum	1 (2)	5.8	(0.9 - 37)	all	all
Podocarpus lawrencei	2 (2)	5.6	(0.9 - 34) #	all	all
Pomaderris apetala	1 (1)	5.4	(0.8 - 34.9)	all	0.96
Senecio linearifolius	1 (2)	5.4	(1 - 29.5)	all	0.92
Olearia argophylla	2 (4)	5.1	(0.9 - 29.3) #	0.74	0.64
Eucalyptus globulus	1 (2)	5	(0.8 - 32.1)	0.93	0.89
Grevillea synapheae	2 (1)	5	(0.8 - 29.7) #	0.92	0.80
Isopogon cuneatus	1 (3)	5	(0.8 - 32) #	0.92	0.76
Prostanthera lasianthos	2 (4)	5	(0.9 - 27.7)	0.97	0.90
Melaleuca squamea	2 (1)	4.9	(0.8 - 29.7) #	all	0.96
Bauera rubiodes	2 (1)	4.5	(0.7 - 26.8) #	all	all
Hakea rostrata	1 (1)	4.2	(0.8 - 22.7) #	0.77	0.66
Taxandria marginata	1 (1)	4.1	(0.6 - 26)	all	all

Correa alba	3 (2)	3.9	(0.6 - 24.6)	0.75	0.72
Correa backhouseana	1 (2)	3.8	(0.6 - 24.9)	all	0.94
Correa decumbens	2 (4)	3.7	(0.6 - 22.4) #	0.93	0.85
Tristaniopsis laurina	2 (4)	3.6	(0.6 - 21.7)	0.86	0.77
Correa reflexa	3 (5)	3.2	(0.5 - 19.2) #	all	all
Corymbia maculata	1 (2)	3.1	(0.4 - 21.7)	0.93	0.92
Leptospermum grandiflorum	2 (1)	3.1	(0.5 - 18.7) #	all	0.98
Eucalyptus delegatensis	1 (2)	3	(0.5 - 19) #	all	all
Lagarostrobos franklinii	2 (3)	3	(0.5 - 17.8) #	0.75	0.58
Acmena smithii	2 (4)	2.9	(0.5 - 17.4)	0.92	0.77
Correa 'Ivory Bells'	2 (2)	2.9	(0.4 - 19.8) #	0.92	0.92
Dicksonia antarctica	3 (2)	2.9	(0.5 - 16.3) #	0.81	0.68
Callitris rhomboidea	2 (2)	2.8	(0.5 - 16.9)	0.35	0.23
Ceratopetalum apetalum	1 (2)	2.7	(0.4 - 17.3)	0.94	0.71
Leptospermum lanigerum	4 (3)	2.6	(0.5 - 14.4) #	0.95	0.87
Tasmannia lanceolata	3 (4)	2.3	(0.4 - 12.5)	0.63	0.49
Tolerant (0 - 2 mm)					
Bursaria spinosa	1 (2)	1.9	(0.3 - 10.3) #	0.85	0.81
Lomatia myricoides	2 (3)	1.6	(0.3 - 9.5) #	0.54	0.52
Adenanthos obovatus	2 (1)	1.5	(0.2 - 8.8)	0.84	0.64
Banksia attenuata	1 (2)	1.3	(0.2 - 8.4)	all	all
Banksia marginata	5 (6)	1.2	(0.2 - 6.5) #	0.99	0.96
Eucalyptus haemastoma	2 (2)	1.1	(0.2 - 6.5) #	all	0.98
Eucalyptus regnans	1 (1)	1.1	(0.7 - 1.9) #	all	all
Leptospermum scoparium	3 (3)	0.8	(0.1 - 4.9) #	0.91	0.85

Pittosporum undulatum	2 (4)	0.6	(0.1 - 3.4)	0.46	0.33
Billardiera heterophylla	3 (4)	0.5	(0.1 - 3.5)	0.44	0.40
Macadamia tetraphylla	1 (1)	0.4	(0.1 - 2.5)	0.86	0.79
Correa 'Sister Dawn'	1 (1)	0.3	(0 - 2.5)	all	0.94
Stylidium graminifolium	1 (1)	0	(0 - 0.3)	all	0.87

[a] Mean predicted lesion length > 30 mm = high susceptibility, 15 – 30 mm = moderate susceptibility, 2 – 15 mm = low susceptibility, 0 – 2 mm = tolerant. Positive control species are known natural hosts of *P. ramorum*.

[b] The number of individual plants (and experiments) for each species. Four to ten branches of each individual plant of each species were tested for each experiment.

[c] Mean (range) predicted lesion length (mm). Range calculated as the addition of the standard error, above and below the predicted mean lesion length. Girdling occurred on some branches (#).

[d] Predicted proportion of branches positively infected with *P. ramorum*, as confirmed by reisolation.

[e] Predicted proportion of branch sections that gave positive reisolation of *P. ramorum*. Non-estimatable prediction with an original mean approaching all branches infected (^).

Table 4. Potential susceptibility and infection potential of six Australian tree species to bole canker diseases caused by *Phytophthora ramorum* 36 to 40 days after wound inoculation with seven different isolates in summer (August 2009), presented in descending order of greatest mean lesion area.

Susceptibility group and species [a]	Trees (logs) [b]	Mean lesion length (cm) [c]		Mean lesion width (cm) [c]		Mean lesion area (cm^2) [c]		Proportion of logs infected [c,d]	Infection potential [c, e]
Positive control species									
High susceptibility									
Notholithocarpus densiflorus	6 (7)	14.7	(1 - 37.4)	5.5	(1 - 10.5)	59.0	(4.8 - 249.7)	0.96 (0.86 - 1)	0.84 (0.71 - 1)
Australian species									
High susceptibility									
Eucalyptus regnans	3 (10)	15.8	(1.8 - 30.4)	4.6	(1.2 - 14)	55.4	(1.2 - 199.3)	0.84 (0.40 - 1)	0.63 (0.28 - 0.80)
Low susceptibility									
Eucalyptus denticulata	4 (13)	5.8	(1.1 - 14.8)	2.3	(0.8 - 5.5)	9.6	(0.8 - 41.7)	0.80 (0.67 - 0.92)	0.49 (0.26 - 0.65)
Eucalyptus globulus ^	9 (14)	4.8	(0.8 - 15.7)	1.7	(0.8 - 4.6)	6.8	(0.4 - 38.9)	0.87 (0.71 - 1)	0.55 (0.26 - 0.65)
Acacia dealbata	9 (10)	5.8	(2.9 - 16.6)	1.3	(1 - 2)	5.5	(2.2 - 15.9)	0.89 (0.70 - 1)	0.59 (0.45 - 0.80)

Tolerant									
Eucalyptus viminalis	9 (12)	4.5	(1.3 - 13.8)	1.7	(1 - 5.9)	4.8	(1.1 - 21.7)	0.82 (0.58 - 0.92)	0.54 (0.39 - 0.69)
Eucalyptus diversicolor	3 (10)	3.2	(1.1 - 8.5)	1.7	(1 - 5.7)	3.7	(1.0 - 12.3)	0.46 (0.20 - 0.60)	0.27 (0.10 - 0.35)

[a] Tolerant species = no significant difference in mean lesion area from negative control inoculations. Where lesion area is significantly different ($P \leq 0.05$) to negative control inoculations and mean lesion area ≥ 20 cm^2 = high susceptibility; ≥ 10 cm^2 to < 20 cm^2 = moderate susceptibility; and < 10 cm^2 = low susceptibility. Positive control species is a known natural host of *P. ramorum*. Composite data of logs collected from two different locations (^).

[b] The number of individual trees (log replications) for each species. Each log was inoculated with seven *Phytophthora ramorum* isolates, one *P. cinnamomi* isolate and a negative control agar plug. Trees were inoculated over three experiments.

[c] Mean (range).

[d] The proportion of logs infected with *P. ramorum*.

[e] The proportion of reisolation of *P. ramorum* as a measure of the number of isolated tissue pieces with *P. ramorum*/the total number of tissue pieces plated for reisolation.

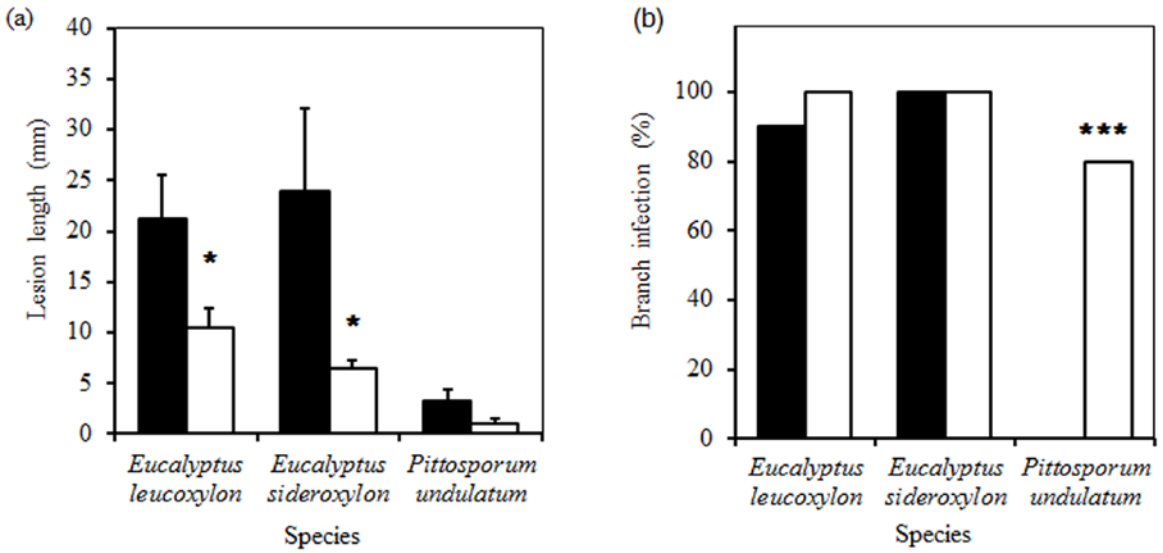

Figure 1. Effect of branch age on susceptibility of *Eucalyptus leucoxylon*, *E. sideroxylon* and *Pittosporum undulatum* as a measure of means ± standard error for lesion length (a) and percent of branches infected (b). Less woody juvenile branches (black) are compared with mature woody branches (white). Asterixes denote significant statistical significance between branch ages, where $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

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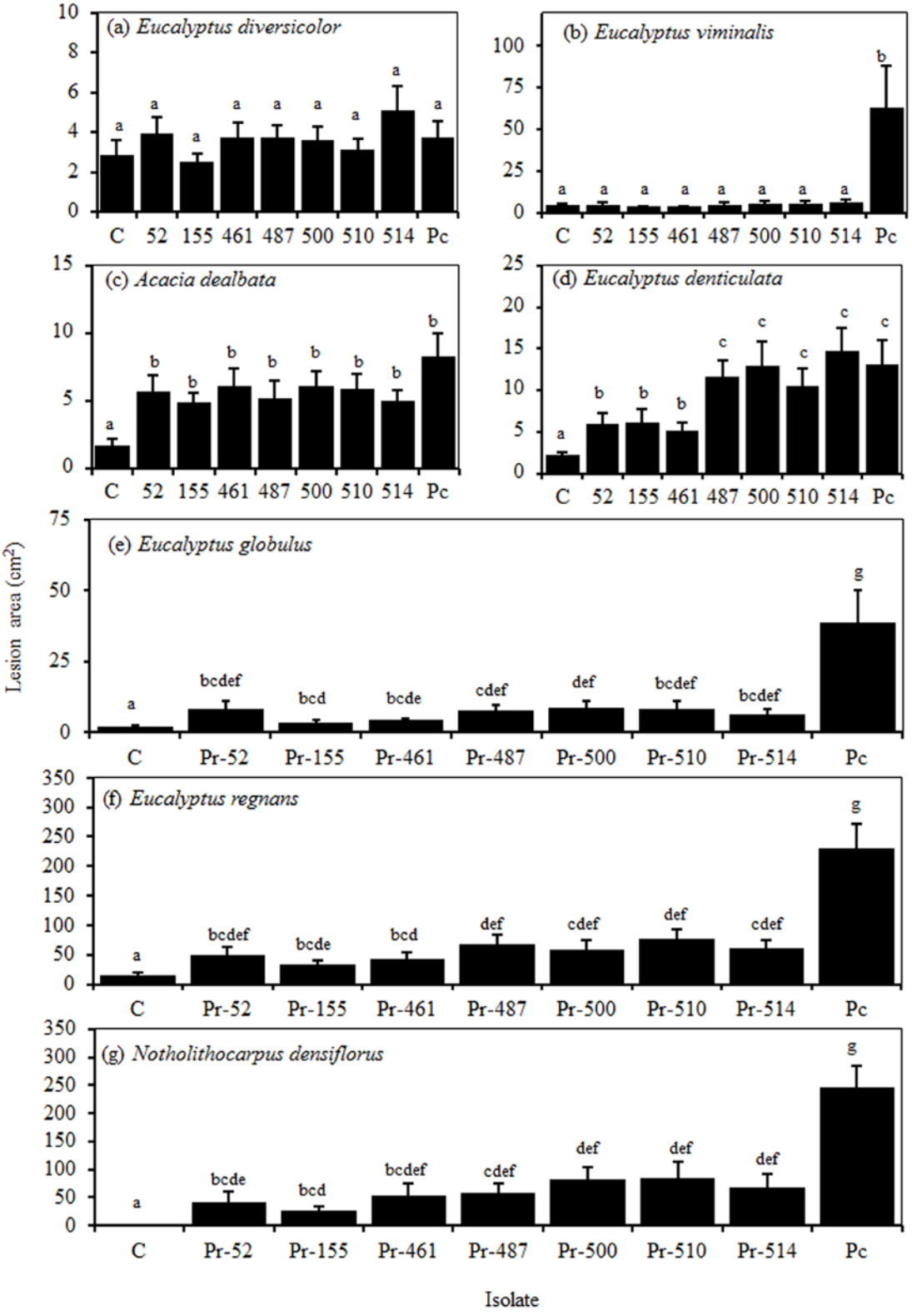

Figure 2. Mean (± SE) lesion area formed on logs 36 to 40 days after inoculation with isolates of *Phytophthora ramorum* (Pr) and *P. cinnamomi* (Pc) on six Australian plants (a-f) and the positive control species *Notholithocarpus densiflorus* (g). C = control. Lesion areas assigned with different letters are significantly different ($P = \leq 0.05$).

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